Supplementary Materials and Methods

Animals

Ten cynomolgus monkeys (*Macaca fascicularis*), aged 5–7 (5.800±0.789) years, were used in this study. The monkeys were singly housed (80 cm×80 cm×80 cm) in a controlled environment (temperature: 22±1 °C; relative humidity: 50±5%) under a 12 h light/12 h dark cycle (lights off at 2000 h and on at 0800 h). The monkey facility is accredited by AAALAC International and all experimental protocols were approved in advance by the Institutional Animal Care and Use Committee of Yunnan Key Laboratory of Primate Biomedical Research (approval ID: LPBR201903003). All monkeys were given commercial monkey biscuits twice a day and fed with fruit and vegetables once daily. The animals were accommodated in their cages for at least one year prior to initial manipulation, and routine veterinary care was provided throughout the experiment to ensure good health by professional keepers and veterinarians.

Video recording

Behaviors of the 10 cynomolgus monkeys were continuously video recorded for seven consecutive nights from 2000 h to 0800 h, using a focal follow technique (Altmann, 1974; Qin et al., 2013, 2015a, 2015b, 2015c, 2016, 2019a, 2019b). The video system consisted of 10 infrared video cameras and a hard disk video recorder. The monkeys were given three days to acquaint themselves with the video system prior to recording. One camera faced one cage, ensuring that the entire cage was visible. In total, 84 h of video recordings were collected for each monkey on separate nights sequentially throughout the seven-day period, and all video recordings were stored on a hard disk for subsequent sleep scoring.

Actigraphy monitoring

Actical Physical Activity Monitors (Respironics, USA) were used for actigraphy monitoring of the 10 monkeys during their natural sleep period. The monitors are water resistant (IEC Standard 60529 IPX7), lightweight (16 g), and small (2.9 cm×3.7 cm×1.1 cm). They use a single internal omni-directional accelerometer that senses motion in all directions, integrates the amplitude and frequency of detected motion, and produces an electrical current varying in magnitude. Therefore, increased intensity of motion produces an increase in voltage. Th Actical system stores the actigraphy data in the form of activity counts. Monkey identity, sex, age, and body weight and length, as well as the start date and time of monitoring, were entered into the Actical system (v3.1.0) before testing. The device was mounted on each monkey's neck with a soft belt and was located in the same position on each monkey to ensure the consistency of the obtained actigraphy data. The actigraphy data were collected at a sampling rate of 32 HZ and in epochs of 15 s (shortest available epoch with these monitors). Each monkey was given three days to acquaint itself with the monitor. Actigraphy monitoring started simultaneously as video recording and lasted for seven days. The actigraphy data were saved in 0.25 min intervals and each minute consisted of four consecutive activity counts. The dark (20:00–08:00 h) activity data were used to score sleep.

Sleep scoring

The video recordings were scored by five raters independently, who were blind to

the experimental design. The five viewers analyzed each night of video recordings simultaneously using standardized behavioral classification, with an inter-rater correlation coefficient of >0.90 obtained through SPSS statistical analysis. Three behavioral states, i.e., wake, transitional, and relaxed sleep, were scored in 1 min epochs (Noser et al., 2003; Qin et al., 2019b). States lasting <30 s were not considered, whereas states lasting between 30 and 59 s were rounded to 1 min. The animals were considered to be awake when locomotion occurred, or when >3 body or limb movements occurred within 1 min. Sleep was scored as transitional when 1–2 body or limb movements occurred within 1 min. Relaxed sleep was scored when the monkeys exhibited no body or limb movements. Sleep duration consisted of all 1 min epochs scored as transitional or relaxed sleep.

Dark activity data were used to score sleep with the same behavioral definitions as used in video analysis. As mentioned previously, the actigraphy data were recorded in 0.25 min intervals and each minute consisted of four consecutive activity counts. A monkey was scored as wake when 3–4 0.25 min activity counts were recorded in 1 min. Transitional sleep was scored when 1–2 0.25 min activity counts were recorded in 1 min. Relaxed sleep was scored when no activity counts were recorded within 1 min.

Data analysis

Data analysis was conducted using SPSS software (SPSS Inc., USA). The normality of data was computed using the Kolmogorov-Smirnov test. Results demonstrated that all data were normally distributed ($P>0.05$). The estimated values obtained by videography and actigraphy analyses, i.e., total amount of time spent in each of the three states (wake, transitional sleep, and relaxed sleep), were paired in all subjects to calculate Pearson correlation coefficients (r). The value of r ranges from –1 to 1, which expresses the strength of the linear relationship between two variables (r close to 1 indicates a strong positive relationship; r of 0 indicates no relationship; r close to –1 indicates strong negative relationship between the two variables). One-way analysis of variance (ANOVA) was used to analyze differences between the two methodologies. The alpha level was set at $P=0.05$, and all P-values were generated using two-sided tests. Data are presented as means±*SEM* (standard error of the mean). To further analyze significant differences between sleep variables, Bland-Altman plots were constructed to examine the agreement between actigraphy and videography. Biases (defined as mean difference between actigraphy and videography sleep variables) for the lower and upper 95% confidence intervals (defined as mean difference±1.96 SD) are presented. A positive mean difference was indicative of overestimation and a negative mean difference was indicative of underestimation.

To further determine an exact percentage agreement between the actigraphy and videography methods, epoch-by-epoch analyses were carried out using PC-developed software based on the same number of 1 min epochs. Videographic analysis was considered as the reference methodology, with rows corresponding to videography and columns corresponding to actigraphy. Thus, 2×2 and 3×3 contingency tables were obtained, and each minute of each state ended up in one of the four or nine cells. Cells on the diagonal (upper left to lower right) indicated agreement between videography

and actigraphy techniques for each state. Other cells indicated the number of incorrectly scored epochs by actigraphy analysis. Moreover, a final table relating to all animals for all night-recordings was obtained by automatic completion. Data were expressed in percentages.

SUPPLEMENTARY REFERENCES

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Supplementary Tables

Supplementary Table S1 Durations of states of sleep based on videography and actigraphy analyses

Monkey	Wake*		Transitional sleep*		Relaxed sleep*	
	Videography	Actigraphy	Videography	Actigraphy	Videography	Actigraphy
Monkey 1	818	46	1 431	244	2 791	4 750
Monkey 2	851	84	1 220	264	2 969	4 692
Monkey 3	1 197	48	1 470	586	2 373	4 406
Monkey 4	1 223	261	1 250	693	2 567	4 086
Monkey 5	787	114	1 107	529	3 146	4 397
Monkey 6	823	37	1 116	292	3 101	4 711
Monkey 7	804	145	1 283	660	2 953	4 235
Monkey 8	921	180	1 612	486	2 507	4 374
Monkey 9	1 222	82	1 810	524	2 008	4 434
Monkey 10	1 263	167	1 661	423	2 116	4 450

Values are presented in minutes. *: Significance level of inter-methodological differences for three states during dark periods, $P<0.05$.

Supplementary Table S2 Epoch-by-epoch analysis for wake and sleep showing total duration (min) and corresponding percentage (%) of epochs scored in agreement (diagonal from left to right) between videography (rows) and actigraphy (columns) methods and those scored differently by actigraphy analysis

State	min		%	
	Wake	Sleep	Wake	Sleep
Wake	1 104	8 805	11.141	88.859
Sleep	60	40 431	0.148	99.852